Finding the needle in the haystack: systematic identification of psychobiotics

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Abstract

The brain-gut-microbiota axis is increasingly viewed as a novel paradigm in neuroscience with the capacity to generate innovative therapies for patients with psychiatric illnesses. Psychobiotics, defined as live bacteria which when ingested in adequate amounts confer mental health benefits, are increasingly of interest, as pre-clinical trials continue to show promising results. Particularly in stress related, anxiety and depressive disorders, there is potential for psychobiotics to deliver new therapies. The question of which microbes may prove to be the most promising psychobiotic in delivering such therapies at clinical level is of great importance. Here we look at the characteristics of psychobiotics, in an attempt to present an outline, from which the identification of potential new psychobiotics may be possible.

Abbreviations

B-GOS-Bimuno- Galacto-oligosaccharides

CCL- Coping Checklist

CRP-C Reactive Protein

CRH-Corticotropin-releasing hormone

FDA-Food and Drug Administration (US)

FMT- Faecal Microbiota Transplant

FOS- Fructo-Oligosaccharides

GABA- gamma-aminobutyric acid

GOS- Galacto-Oligosaccharides

HADS- Hospital Anxiety and Depression Scale

HMOs- Human Milk Oligosaccharides

HPA- Hypothalamic-Pituitary-Adrenal axis

HSCL-90- Hopkins Symptom Checklist

IL-6- Interleukin 6

IL-10- Interleukin 10

IL-2-Interleukin 2

Il-1-Interleukin 1

IBD- Inflammatory Bowel Disorders

IGA- Immunoglobulin A

IGM- Immunoglobulin M

IND-Investigational New Drug

MAMPs- Microbe Associated Molecular Patterns

SCFAs- short chain fatty acids

TLR -Toll Like Receptor

TNF-α -Tumour necrosis factor alpha

UFC- Urinary Free Cortisol

WHO-World Health Organisation

Gut-Brain-Microbiota Axis

The brain-gut-microbiota axis consists of several key components, the central nervous system, the neuroendocrine system, the neuroimmune system and most importantly the gut microbiota. The gut microbiome is composed of all microorganisms and their genomes, in the intestinal space. Bacterial concentrations in the gut range from 10^1-10^3 per gram in the upper intestines to 10^{11}-10^{12} in the colon (O'Hara and Shanahan 2006). The complex interaction between these components operates in a bi-directional communication network between the brain and the gut. Evidence continues to demonstrate that the gut microbiota has significant impact on brain function (Stilling, Dinan et al. 2014). Many studies have now demonstrated that the gut-brain-microbiota axis contributes to the regulation of brain physiology and ultimately behaviour. Various signalling pathways have been suggested, all of which demonstrate the link between brain modulation, behaviour and the gut microbiome (Cryan and Dinan 2015).

The term probiotic was first introduced by Metchnikoff in 1908. Probiotics today are defined as a live organism that when ingested in adequate amounts, exerts a health benefit (Dinan

and Quigley 2011). However, there is an ongoing need to revise and refine this definition as more research is conducted in this area. For example, it has been demonstrated that even dead probiotic microorganisms can induce immune reactions when ingested in adequate amounts (Dinan, Stanton et al. 2013). Specific probiotics appear to have benefits in particular disease states and disorders.

Prebiotics are indigestible food material, for example oligosaccharides, with selective fermentation in the colon, which affects the microbiome by inducing growth, activity or both, of one or several bacteria in the microbiome (Roberfroid, Gibson et al. 2010). Prebiotics are of interest as several studies have confirmed certain prebiotics can alter the gut microbiota to reduce low grade inflammation (Bindels, Delzenne et al. 2015). They can also inhibit the growth of pathogenic bacteria. Synbiotics, are described as "mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare" (Roberfroid, Gibson et al. 2010). Probiotic preparations, containing multiple strains of probiotics have also shown promise in disorders such as ulcerative colitis (Wasilewski, Zielinska et al. 2015). These "polybiotic" preparations may prove of significant interest in the future development of psychobiotics.

Since the term Psychobiotics was first introduced in 2012 and defined as "a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness" (Dinan, Stanton et al. 2013) the search for new and novel therapeutics in this area continues. The term psychobiotics has also been expanded to include prebiotics (Sarkar, Lehto et al. 2016) and may feasibly confer benefits to both in the patient population and the at risk population. Preclinical studies demonstrating the effects of the gut microbiome on neuroimmune, neural and hormonal pathways the suggestion is that the gut microbiota can be modulated to treat neuropsychiatric disorders, or at the very least offer adjunctive treatment options for these disorders (Cryan and Dinan 2015, Kelly, Clarke et al. 2016). Although much has been made of stress related psychiatric disorders, such as depression and anxiety, as the exploration of the complex and not fully discovered relationship between the brain-gut-microbiota axis continues, there is enormous scope for psychobiotics as therapeutics in patients suffering from a wide variety of psychiatric illnesses.

In the search for psychobiotics, previous studies have focussed on strains of bacteria secreting gamma-aminobutyric acid (GABA), tryptophan and short chain fatty acids (SCFAs) or

impacting the hypothalamic-pituitary-adrenal axis (HPA) and the inflammatory response pathway (Jokela, Hamer et al. 2014). All of these parameters have shown anomalies in stress and especially in depression and anxiety disorders.

How do we identify a beneficial bacterium for neuropsychiatry treatment? How do we set out to discover potential new psychobiotics? In this review we discuss the attributes of known psychobiotics and the features which provide benefits for hosts. We discuss how these benefits are known to affect the brain-gut-microbiota axis. If new putative psychobiotics are identified how can we know which might work and allow us to focus only on trials most likely to succeed? Here we propose a strategy for the identification of novel psychobiotics.

Brain-gut-microbiota axis abnormalities in depression and related disorders

Depression is an intricate mood disorder affecting 121 million people worldwide. WHO predicts an increase to elevate depressive disorders second only to cardiovascular disease by 2020 in terms of total disease burden worldwide. Depression is often reoccurring, frequently has comorbidity with anxiety and is associated with significant disability worldwide (Kelly, Clarke et al. 2016). Depression results in dysregulation of neuroendocrine, neuroimmune, neurotransmitter and endocrine functions (Jokela, Hamer et al. 2014). A feature of depression which is repeatedly demonstrated is the low-grade inflammatory response observed in this patient population (Kelly, Borre et al. 2016). Specific pro-inflammatory biomarkers have been identified as being of significance in depression including raised Interleukin (IL)-6, Tumour necrosis factor alpha (TNF-α) and C-Reactive Protein (CRP) (O'Brien, Scott et al. 2004). Such is the impact of certain pro inflammatory cytokines such as interferon-α, that injection with this cytokine is known to induce a depressive episode (McNutt, Liu et al. 2012). Stress can influence the development of the intestinal barrier and has been linked with increased permeability of the gut (Moussaoui, Braniste et al. 2014). Kelly, Borre et al. (2016) have recently shown a significant reduction in microbiota richness and diversity in those suffering with depression. Furthermore, transferring the altered gut microbiota to germ-free rats induced a depressed-phenotype in these rats. This study suggests that the gut microbiota may play a causal role in depression pathophysiology.

Genomic identification of psychobiotics

The most probable method in which to quickly and efficiently identify potential new psychobiotics is to fully characterise and analyse individual bacteria currently known to have beneficial effects. Individual bacterial strains with various probiotic properties should be assessed to unveil the genetic description of those with beneficial phenotypes. This has begun in earnest with several recent studies describing whole genomes of various probiotics (Arnold, Monteagudo-Mera et al. 2017). Mapping of the entire gut microbiome has revealed most genes to be bacterial in origin (Qin, Li et al. 2010). Metagenomic sequencing approaches to evaluate the microbiome are useful, however it should be enhanced with proteomic and metabolomic analysis to determine which microbial genes and proteins are expressed in various conditions (Tremaroli and Bäckhed 2012). Such inter-omic analyses allows for the investigation and characterisation of the gut microbiota ecosystem. Work to date has unveiled various genetic traits, including horizontal gene transfer in some strains of *Lactobacillus.* (Douillard, Ribbera et al. 2013). Bile resistance, and anti-microbial activity have all been identified in studies in the microbiota (Siezen and van Hylckama Vlieg 2011). Further characterisation will allow for a genetic signature to be built in order to identify the most appropriate psychobiotics for those suffering with psychiatric conditions. Such an approach should hasten discovery and help eliminate unsuitable microbes.

Studies have highlighted the relationship between the gut microbiome and the mucosal proteome (Presley, Ye et al. 2012), indicating that bacterial composition of the microbiome may alter the mucosal proteome. McHardy, Goudarzi et al. (2013) demonstrated the importance of considering the proteome, metabolome and the genome concurrently. The inter-omic play between the microbiome and the mucosa is of upmost importance and should not be discarded when considering the complexities of the gut brain axis, and the potential for probiotics to alter the axis in various ways. Noecker, Eng et al. (2016) presented a framework to take into account all "omic" interactions, in an attempt to evaluate the microbiome at a multi-omic, multi-system level, rather than evaluating all aspects individually. This framework may allow for better understanding of all factors affecting the microbiome and the complex multidimensional interactions at play. It is likely that the genomics of both the host, the composites of the microbiome, and the proteomic and metabolomic composition will also have a significant role in furthering our understanding of disease states.

Anti-inflammatory action and immune response and its role in Psychobiotic identification

The microbiome interacts with the gut epithelial surface to trigger immune responses and in early life plays a key role in shaping the neuroimmune evolution (O'Hara and Shanahan 2006). In particular Toll Like Receptors (TLRs) which recognise various structural components of bacteria can trigger pro-inflammatory responses (McCusker and Kelley 2013). An altered gut microbiome in early development can predispose to such varied conditions as inflammatory bowel disease (IBD), increased stress reactivity, and predisposition to anxiety and depression (Dinan, Stanton et al. 2013). Most probiotics tested have anti-inflammatory action which may be beneficial to a multiplicity of states, including such diverse diseases as IBD, post-operative states and psychiatric disorders such as anxiety and depression. Dai, Zheng et al. (2013) demonstrated that certain probiotics induce IL-10 anti-inflammatory response, and down-regulate a variety of inflammatory drivers including TNF-α and IL-6. IL-6 together with IL-2, IL-1 & TNF-α are hallmarks of a depressive disorder and are well characterized and readily identifiable in this affective state.

Probiotic activity has been shown to triggers immune responses stimulating the anti-inflammatory pathways based on bacteria genera and Microbe Associated Molecular Patterns (MAMPs) (Mackey and McFall 2006). MAMPs are thought to trigger such anti-inflammatory cytokines as 1L-10 by stimulating pattern-recognition receptors (Chu and Mazmanian 2013). In particular, some beneficial bacteria such as *Bifidobacteria*, appear to prevent TLRs being activated and initiating a pro-inflammatory response (Zhou, Lv et al. 2015). Prebiotics and probiotics have been shown to interact with TLRs initiating both pro and anti-inflammatory responses (Takeda and Akira 2005). There is mounting evidence that prebiotics prevent MAMPs triggering immune responses. This mechanism is most likely occurring through direct interaction on the gut epithelium with oligosaccharides (Sarkar, Lehto et al. 2016). Furthermore, the mere presence of probiotics can have an anti-inflammatory effect. Most are gram positive and lack lipopolysaccharide (LPS), reducing pro-inflammatory responses as they colonize the gut (Gayathri and Rashmi 2017). Neonatal studies have shown that the prebiotic Human Milk Oligosaccharides (HMOs) are particularly adept at inhibiting pro-inflammatory states (Wickramasinghe, Pacheco et al. 2015). It seems reasonable to assume that bacteria which induce an anti-inflammatory response are likely to show psychobiotic activity.

The Microbiota and Intestinal barrier permeability

The gut barrier has a pivotal role in defending the body through its epithelium, by maintaining pathogens and toxins whilst regulating the absorption of nutrients. The microbiota composition has an intricate relationship with the permeability of the epithelial membrane, with the microbiota and its metabolites having a role in altering the permeability of the membrane (Jakobsson, Rodriguez-Pineiro et al. 2015). Increased permeability of the intestinal epithelium has been associated with a pro-inflammatory state (Kelly, Clarke et al. 2016). In response to an acute stress the colonic paracellular permeability has been shown to increase and can be linked to both inflammatory responses and translocation of the gut microbiota (Moussaoui, Braniste et al. 2014). Rat pups subjected to early life stress in the form of maternal separation, demonstrated altered gut microbiota, increased plasma corticosterone and an overall increase in systemic immune response (O'Mahony, Marchesi et al. 2009). This may increase susceptibility to pro-inflammatory diseases such as depression and anxiety in the future. Increased permeability of the intestinal barrier allows certain gram negative bacteria components to translocate from the gut and trigger pro-inflammatory pathways (Lucas and Maes 2013). This is the suspected mechanism of action for the inflammatory state observed in depression. Higher immunoglobulin (IgA, IgM) mediated immune responses to these gram negative bacteria are also observed in depression (Maes, Kubera et al. 2013).

Lactobacillus helveticus R0052 has demonstrated its ability to reduce intestinal permeability as a result of a barrier effect (Messaoudi, Lalonde et al. 2011). Kelly, Borre et al. (2016) have shown reduced gut microbiota diversity at phyla level in faecal microbiota transplant (FMT) rats from depressed patients. This shows the significant alteration in microbiota in neuropsychiatric stress related disorders. The study also showed increased CRP in plasma of FMT rats revealing a pro-inflammatory response at systemic level related to the altered microbiota. It can be reasonably surmised that the plasma response was secondary to altered epithelial permeability as a result of dysbiosis following FMT from depressed subjects. If intestinal permeability is compromised the ensuing immune response could trigger an array of neuropsychiatric responses. Recent studies have highlighted the role of prebiotics in enhancing the microbiota present in the gut. Particular interest is arising in Fructo-oligosaccharides (FOS) and Galacto-oligosaccharides (GOS). A recent study by Holscher, Bode et al. (2017) has demonstrated that administration of HMOs enhance gut epithelial differentiation and maturation. This study strengthens the theory that oligosaccharides

directly affect the epithelium and by preventing a 'leaky gut' minimise pro inflammatory responses. There is potential to use psychobiotics to target the gut epithelium and to reduce permeability in an effort to maintain integrity and reduce immune responses.

Hypothalamic-Pituitary-Adrenal axis (HPA) and the stress response

The HPA circuit is long recognised as an important regulatory loop between brain and gut. Hyper-activation of the HPA has been been identified in various psychological and affective states, including depression anxiety and stress (Flandreau, Ressler et al. 2012). However, the question of whether this affective state triggers over-activation of the HPA, or whether hyperactivity of the HPA results in the affective state has been the subject of some debate. The primary hypothalamic regulatory peptide is Corticotropin-releasing hormone (CRH), in response to psychological stress it is released by neurotransmittors such as norepinephrine and 5-HT (Dinan and Cryan 2012). Studies evaluating the altered HPA responses in patients with both major depression and irritable bowel disease indicate this may be induced by increased gut permeability (Dinan and Cryan 2012). The use of germ free animals has exposed interesting findings in the role of the microbiota and the development of the HPA. In the absence of microbiota in the gut, TLRs have reduced expression in gut epithelia and thus they are not present to initiate the immune cascade which results in activation of the HPA (Gosselin and Rivest 2008). The stress response in germ free animals results in exaggerated release of corticosterone, a response fully reversed by monoassociation with *Bifidobacterium infantis* (Kelly, Clarke et al. 2016). The role of the microbiota in the development of appropriate stress responses and the development of the HPA is demonstrated in this study. A microbiota demonstrating abilities to reduce a stress response is most clearly showing psychobiotic potential. An ongoing question is how best to measure a stress response? Many endocrine studies utilise salivary or plasma cortisol sampling, other studies measure various cytokine markers. A consensus has yet to be reached.

Short chain fatty acids (SCFAs) and psychobiotic potential

SCFAs are the metabolites of indigestible macronutrients derived from for example plant polysaccharides. The administration of prebiotics increases SCFA production strengthening this concept (Psichas, Sleeth et al. 2015). The enzymes to digest such polysaccharides are supplied in the human gut by the microbiome (Qin, Li et al. 2010). SCFAs have increasingly

been investigated for potential psychobiotic therapy. The SCFAs include butyrate, acetate and propionate. Butyrate in particular, has been demonstrated to have antidepressant properties in a number of studies. It has the ability to cross the blood brain barrier, and has been shown to have numerous neuroprotective abilities, with demonstrated cognitive and antidepressant properties (Han, Sung et al. 2014). Further support as to the antidepressant effects of butyrate were demonstrated by Moretti, Valvassori et al. (2011) who showed reduced stress behaviours and antidepressant effect following systemic butyrate injections in rats. This action is in keeping with butyrate as an epigenetic modulator via its regulation of gene expression through inhibition of histone deacetylase (Stilling, Dinan et al. 2014). It has been noted that SCFAs directly affect the mucosal immune system, can alter the HPA axis and through these mechanisms appear to alter the central neurotransmission (Perry, Peng et al. 2016). Butyrate's ability to act as a neuroprotective agent together with its effects on memory and cognition is of particular interest given that loss of cognitive abilities is a long recognised and undertreated feature of recurrent and severe depressive disorders. As our understanding of SCFAs expands, their potential use as therapeutics becomes more tangible, and we foresee a role for SCFAs in the evolution of psychobiotics.

Tryptophan metabolism

Tryptophan is an essential amino acid that must be ingested in the diet. The brain has limited tryptophan storage capacity and consequently a constant supply is required. Certainly reduced peripheral levels of tryptophan are associated with a depressive phenotype (Ogawa, Fujii et al. 2014). The brain-gut-microbiota axis and tryptophan metabolism are inherently linked, with tryptophan levels and availability influenced directly by the gut microbiota (Desbonnet, Garrett et al. 2008). Bacteria such as *Bifidobacteia* have been shown to increase peripheral tryptophan levels (Desbonnet, Garrett et al. 2008), and certainly have psychobiotic potential. The majority of serotonin is synthesised in the gut by enterochromaffin cells from tryptophan and bacteria are thought to play a role in this metabolism (Clarke, Grenham et al. 2013). Specific microbiota metabolites have been shown to increase colonic and plasma serotonin levels (Kelly, Borre et al. 2016) and in germ free mice, levels of serotonin increase almost three fold when colonised by gut microbes (Clarke, Grenham et al. 2013). *Bifidobacteria,* with the ability to raise peripheral tryptophan are a true target for psychobiotic development.

Diet and mental illness

Diet in a healthy adult is the main determinant of gut microbiota composition. The association between diet and psychiatric disorders, particularly depression and anxiety has long been suggested and gut microbes may be the important linking factor. Association between the Mediterranean diet and reduction in depression is well established. The Mediterranean diet is defined as being high in plant foods (fruit, vegetables, breads, cereals, potatoes, beans, nuts, and seeds), fresh fruit, olive oil, moderate amounts of dairy products (cheese and yogurt), and fish and poultry, zero to four eggs consumed weekly, red meat consumed in low amounts, and wine consumed in low to moderate amounts, traditionally with meals. Even moderate adherence to the Mediterranean diet results in reduced risk of depression (Psaltopoulou, Sergentanis et al. 2013). Other studies have demonstrated that a more "Western diet", defined as a diet high in processed or fried foods, refined grains, sugary products, and beer, are associated with higher reported levels of anxiety and depression in women (Jacka, Pasco et al. 2010). Analysis of the microbiota in those on a Mediterranean diet may help identify bacterial strains with psychobiotic activity.

What behavioural studies in rodents help to identify psychobiotics?

A number of well validated rodent behavioural tests are used to assess the effects of altering gut microbiota on such mental states. The rodent response in such tests allows for inference of effects from specific bacteria. In approach-orientation, time spent exploring new environments is a key behavioural feature, as it is crucial for the rodent to do so in order to adapt to novel and changing environments. Stress and anxiety are shown to significantly reduce exploration behaviours. The forced swim test in rodents, is based on progressive immobility displayed by the rodent when forced to swim in a container of water. Immobility in the forced swim test has long been associated with increased anxiety and depressive behaviours in rodents. The test has frequently been used to identify the antidepressant potential of drugs, and now psychobiotics.

Germ free mice demonstrate reduced anxiety by showing more exploratory behaviours in approach orientation tests (Diaz Heijtz, Wang et al. 2011). However once germ free mice are exposed to stress they exhibit elevated and exaggerated glucocorticoid responses, and reduced exploratory behaviours (Desbonnet, Clarke et al. 2015). Proving beneficial in

examining how the gut microbiota may alter rodents behavioural phenotype is the use of faecal transfer of the microbiota of interest to germ free rodents. Bercik, Denou et al. (2011) have shown that transferring the microbiota from stress-sensitive mice strains to non-anxious mice strains, can alter phenotype and reduce exploratory behaviours inferring increased anxiety in the recipient mice.

Testing of potential psychobiotics using rodent behavioural tests is an important step in psychobiotic identification.

Prebiotics, "Polybiotics" & Synbiotics as Psychobiotics

Prebiotics have been defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Roberfroid, Gibson et al. 2010). Studies have shown prebiotics may have an ability to survive for extended periods in the gut, and one study has shown potential to promote longstanding benefits up to a year after administration (Oliveros, Ramirez et al. 2016). This makes prebiotics increasingly attractive as vehicles for modulating the brain-gut-microbiota axis.

The probiotic "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" has a transient modulating effect on the gut microbiota. Multiple studies show improved functionality which is quickly lost on withdrawal of the probiotic. For example, significantly there is reduced anxiety in mice within 1 week of probiotic treatment, with no effect observed at 3 weeks post treatment (Matthews and Jenks 2013). Patients with IBD report an emergence of symptoms within days of discontinuation of probiotics.

A modified GOS (Bimuno-GOS or B-GOS) and FOS has been shown to have neuroprotective properties in animal models. With administration of B-GOS showing clear evidence of increasing availability of brain derived neurotrophic factor (BDNF) in the hippocampal region (Savignac, Tramullas et al. 2015). There is also evidence of increased cognitive abilities particularly in the area of memory observed following increased BDNF levels (Williams, Chen et al. 2016). Other studies involving B-GOS and FOS have established that the administration of B-GOS significantly lowers the salivary cortisol response (Schmidt, Cowen et al. 2015) . B-GOS reduces anxiety and expected cytokine stress responses in B-GOS fed mice. This study indicates that B-GOS exerts its anxiolytic effect through modulation of IL-1β and 5-HT2A (Savignac, Tramullas et al. 2015). Human studies

have also demonstrated benefits from B-GOS in emotional processing (Schmidt, Cowen et al. 2015). All of which further supports the potential role of oligosaccharides and in particular B-GOS as a potential psychobiotics, whether alone or as adjunctive therapy in affective and anxiety disorders.

Lactobacillus helveticus R0052 and *Bifidobacterium longum* R0175 demonstrate anti-inflammatory properties on intestinal epithelial cells (Messaoudi, Lalonde et al. 2011) and poly-psychobiotics administration of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 concurrently have shown good evidence of reducing depressive behaviours in mice post Myocardial Infarction (Arseneault-Breard, Rondeau et al. 2012), while showing the ability to reduce anxiety levels in healthy volunteers and 24 hour urinary cortisol output. The recurrent difficulties with these studies is that probiotics, whether in multiple strain or single strain, are transient and do not colonise the gut permanently.

Increasingly of interest is synbiotics, a prebiotic co-administered with probiotic. Some studies have demonstrated good use of synbiotics in IBD (Wasilewski, Zielinska et al. 2015) with significant reductions observed and maintained in TNF-alpha for up to 6 months post treatment.

VSL #3 is a probiotic preparation or formula composed of eight live freeze-dried bacterial species that are normal components of the human gastrointestinal microflora, including four strains of lactobacilli (*Lactobacillus casei, L. plantarum, L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*), three strains of bifidobacteria (*Bifidobacterium longum, B. breve* and *B. infantis*) and *Streptococcus salivarius* subsp. *thermophilus*. VSL #3 has demonstrated good results for patients in conditions such as ulcerative colitis and irritable bowel syndrome (Wasilewski, Zielinska et al. 2015). This probiotic preparation could be considered a "polybiotic" and there is the potential to co-administer probiotic formula in the search for psychobiotic therapies. The potential for synbiotics, the amalgamation of pro and prebiotics, is increasingly of interest in the search for psychobiotics. So far no major studies of synbiotics in humans with mental health disorders have been reported.

There may too be a role for multi-strain probiotics or cocktails for pro and prebiotics to be administered in order to confer less transient and more long term benefits. There is as yet no consensus on whether psychobiotic potential is achieved best with a single strain probiotic, a multi-strain "polybiotic" or as a synbiotics, a prebiotic co-administered with probiotic.

Discussion

Determining the preclinical markers that best identify psychobiotics that will benefit patients with depression and related disorders is a major challenge. Only by adequate preclinical evaluation coupled with good clinical studies will effective algorithms emerge for identifying likely psychobiotics. Not all prebiotic, probiotic or synbiotics will have psychobiotic potential, and not all that show preclinical promise will be suitable for further development.

In investigating and developing psychobiotics it is important to focus on those strains that have shown effects on behaviour, gut permeability, are neuroactive, and reduce pro inflammatory and stress responses in pre-clinical studies. Even this strategy will have difficulties however. For example, *Lactobacillus rhamnosus* strain JB-1, demonstrated an ability to reduce stress-related behaviour, corticosterone release and alter central expression of GABA receptors in an anxious mouse strain (Bravo, Forsythe et al. 2011). *Lactobacillus rhamnosus* was therefore predicted to have good potential as a psychobiotic. However, these promising pre-clinical findings, did not translate in healthy male volunteers. There was no difference between *Lactobacillus rhamnosus* and placebo in an 8 week trial in healthy volunteers with a crossover design (Kelly, Allen et al. 2017). This highlights the need for preclinical trials to be moved quickly forward to interventional studies in populations with anxiety, depression and stress related disorders.

Other more promising progress has been made. A double-blind, placebo-controlled, randomized study was conducted on volunteers with symptoms of stress. Subjects received a probiotic (Probio-Stick; Lallemand SAS, Saint-Simon, France) containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R00175 (3×10^9 colony-forming units per sachet stick) or an identical placebo without probiotics for a 3-week period. Though this study showed reductions in stress related abdominal discomfort, it did not have any effect on other symptoms of stress, for example sleep. A further follow up double blind randomised control study by of the same preparation delivered for 4 weeks, showed significantly reduced stress levels in volunteers as recorded using various stress measurement tools (Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL) and a 24h urinary free cortisol (UFC) collection. In 2016, on foot of both studies Lallemands's Probio'Stick® (*Lactobacillus helveticus* R0052 and Bifidobacterium longum Rossell®-175 in a stick presentation) has been accepted by the Canadian Health Authorities for benefits in the area of stress, anxiety and mood.

Personalised psychobiotics may have a role in the future. Many researchers have now recognised that though identical bacterial species colonise the human gut, varying genetics, life experiences and exposures alter the composition of the gut microbiota. This may affect the clinical presentation of the individual in various affective or stress related disorders. This will alter the best "prescribed" probiotic required to address the individual's anxiety or depression. Presently however, the priority lies in identifying generic psychobiotics beneficial to many patients, prior to individualisation becoming a reality.

As research continues the more fundamental the gut microbiota appears to be in affecting the entire system, at almost all conceivable levels. In order to confer the most benefit from potential psychobiotics and develop future therapeutics continuing to evaluate the manner in which they are altering the gut microbiota is of upmost importance. The ability of potential psychobiotics to act on the areas outlined here will allow researchers to target trials on probiotics, probiotic formulations, prebiotics or synbiotics showing preclinical promise and avoid wasting valuable cost and time on unlikely candidates. A targeted and predictive approach may, hopefully, allow the development of psychobiotics in a timely fashion.

Nomenclature of targets and ligands

Key protein targets in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY(Southan, Sharman et al. 2015), and are permanently archived in the Concise Guide to Pharmacology 2017/2018 (Alexander, Fabbro et al. 2017).

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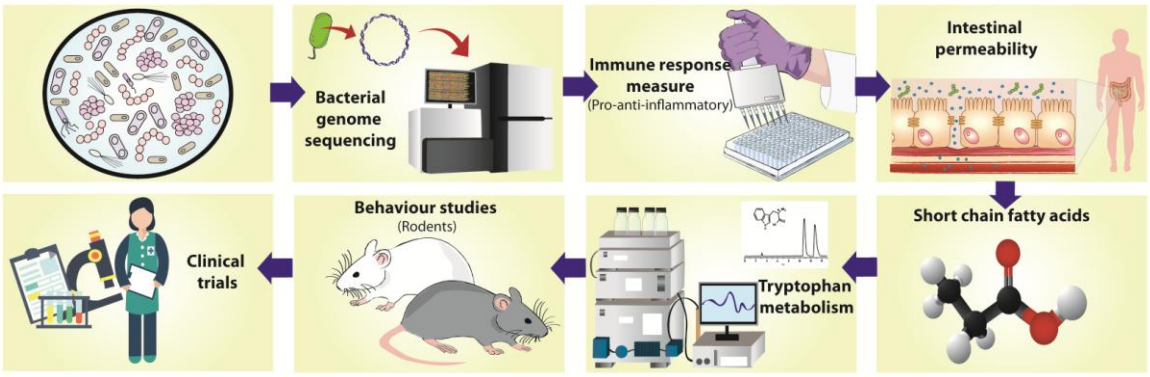

Figure 1. The search for Psychobiotics. This illustrates the potential pathway for psychobiotic identification. The first step in the process is genome sequencing and comparison with strains know to have psychobiotic potential. Determining the potential to generate short chain fatty acids and tryptophan is a fundamental step in the process, as is the ability to demonstrate anti-inflammatory activity and impact on intestinal barrier function. Behavioural studies in animals precede any human intervention studies

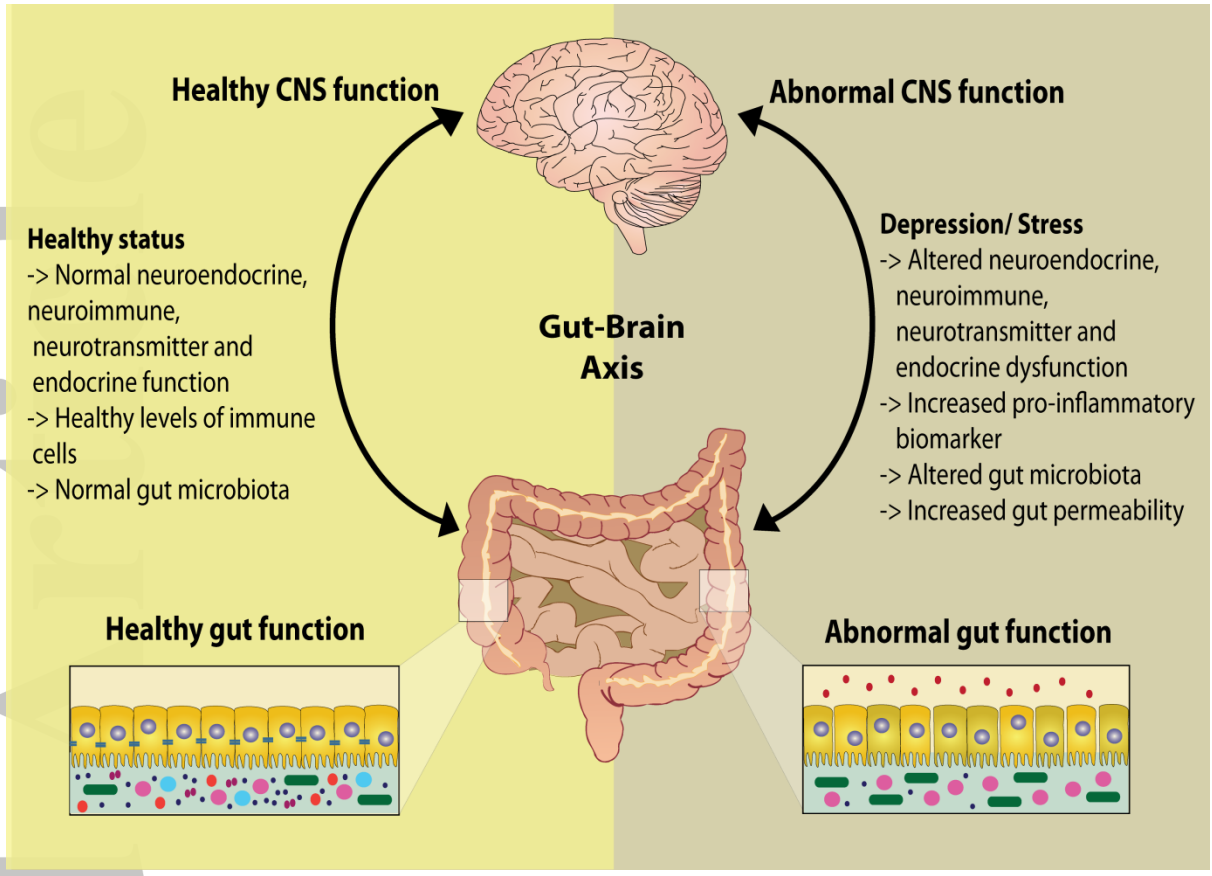

Figure 2. The gut brain axis. Comparison between a healthy gut brain system and an abnormal system. In negative affective states such as stress anxiety or depression, pro-inflammatory markers increase, gut microbiota and gut permeability is altered and the gut brain axis is in a state of dysbosis.